Dietary benfotiamine in high carbohydrate diet improves growth and resistance to abrupt shift to higher salinity in the African catfish *Clarias gariepinus* juveniles

[1]Rey L. Obeda, [2]Barry L. M. Tumbokon, [1,2]Augusto E. Serrano Jr.

[1] Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines; [2] National Institute of Molecular Biology and Biotechnology, University of the Philippines Visayas, Miagao, Iloilo. Corresponding author: A. E. Serrano Jr., serrano.gus@gmail.com

Abstract. Benfotiamine, a synthetic provitamin B1 and a more bioavailable form, has been demonstrated to enhance growth and immune response in fish and shrimps in high carbohydrate diets. The present study aimed to determine the effects of elevated dietary carbohydrates and benfotiamine supplementation on the fry of African catfish *Clarias gariepinus*. Three diets were used, namely: a commercial diet specific for catfish was used as the control (C) diet, a high carbohydrate (HC) diet, and a benfotiamine-supplemented HC (HCB) diet. Results show that the HCB diet resulted in significantly higher final average body weight (FABW), weight gain (WG), specific growth rate (SGR), and significantly better food conversion ratio (FCR) than the values for both C and HC groups. In a separate test for attractability, it was demonstrated that HCB was more attractive to the catfish juveniles than the HC or C diets. Also, an experiment to determine the median lethal concentration after 96 h (96 h LC50) of catfish fry in various salinity levels was conducted and was found to be at 10 ppt salinity. This salinity level was used for the resistance test to abrupt exposure to higher salinity to ascertain whether benfotiamine renders protection in the catfish fry. The results show that catfish fry subjected to abrupt salinity change from 0 to 10 ppt following feeding with the HCB diet exhibited the lowest cumulative mortality, while those fed the C and HC diets showed higher cumulative mortality. In conclusion, elevating dietary carbohydrates in the diet (i.e., C to HC diet) negatively affected the growth of the African catfish. Further supplementation of benfotiamine to the HC diet at 0.02% enhanced significantly ($p<0.05$) both the growth and resistance to sudden shift in salinity from 0 to 10 ppt.

Key Words: attractability, cumulative mortality, hypersalnity test, lethal concentration, provitamin B1.

Introduction. Thiamine, also known as vitamin B1 or aneurine, is a water-soluble essential vitamin present commonly in cells as non-phosphorylated thiamine (T), thiamine monophosphate (TMP), and thiamine diphosphate (TDP) (Marcé-Grau et al 2019). Its active form is thiamine diphosphate (TDP), a major coenzyme involved in several pathways in energy production, including pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, branched-chain α-ketoacid dehydrogenase complex, and transketolase required for mitochondrial oxidative decarboxylation in the pentose phosphate pathway (NRC 2011). Benfotiamine is a synthetic pro-vitamin B1 and a lipid-soluble derivative of thiamine. Compared to the water-soluble form (i.e., thiamine), it has a unique open thiazole ring that enables it to enter directly through the cell membrane, thus leading to a higher rate of bioabsorption and bioavailability of at least five fold higher plasma concentration than that of thiamine in plasma (Schreeb et al 1997; Portari et al 2013; Xie et al 2014). Through oral administration, optimum dosage of thiamine leads to an increase thiamine related derivatives including thiamine monophosphate and thiamine diphosphate in the blood and liver, which catalyze several biochemical reactions resulting in extracting and storing energy from glucose (Oh et al 2009; Fraser et al 2012; Chung et al 2014).

In aquatic animals, the effect of benfotiamine has been studied in terms of growth and immune response in fish and crustaceans (Lauzon et al 2019; Nudalo et al 2020).

Lauzon et al (2019) elucidated that 0.02% benfotiamine supplementation was optimal in enhancing the growth performance of Nile tilapia (*Oreochromis niloticus*). It was demonstrated that blunt snout bream (*Megalobrama amblycephala*) fed high carbohydrate diet supplemented with benfotiamine enhanced growth performance (Xu et al 2017; Lauzon et al 2019). In post-larval *Penaeus monodon* challenged for 72 h, it was demonstrated that dietary benfotiamine at 0.02% concentration enhanced the immune response against ammonia toxicity (Nudalo et al 2020).

African catfish (*Clarias gariepinus*) is an omnivorous fish mainly cultured locally and commercially in wide parts of the world (FAO 2010; Okomoda et al 2017). This catfish is fast-growing and can reach up to 80-300 g in about 3-5 months of culture (FAO 2010; Gonzales 2019). Due to increased demand in the local markets, the imported African catfish is dominantly cultured compared to *Clarias macrocephalus*, despite its invasive behavior (DABPO 2021). This fish has been previously reported to be widely introduced in the Philippines and described to occur in high abundance in local ponds, rice fields and water bodies with slightly higher salinities. In the Philippines, *C. gariepinus* had excellent prices in the local markets and its production had greatly increased (FAO 2010). In 2017, the catfish demand continued to increase with a volume of aquaculture production by species of 4147.26 metric tons, reaching 5420.77 metric tons in 2020 (PSA 2022). There are several operating hatcheries in the Philippines and yet demand still outweighs supply due to ineffective culture management and practices. One of the constraints in catfish production is the poor quality of fry, which contributes to erratic annual yield caused by a higher rate of mortality, particularly during nursing of fry. Survival rate from fry to juvenile ranges between 5% to 80% depending on the fry quality. Moreover, consistency of production during harvest is difficult to maintain (Gonzales 2019). The type of diet plays a crucial role for a sustainable production of *C. gariepinus* at their early stages of life. Weaning of early stage catfish fry to dry diets is vital to their growth and survival.

Shahoo et al 2003 studied the effect of salinity on survival, feed intake, and growth of *Clarias batrachus* fingerlings. The fingerlings were subjected to different levels of salinities (2, 4, 6, 8, 10 ppt). They have reported that water salinity beyond 4 ppt was detrimental to *C. batrachus* fingerlings whose survival and growth was significantly affected. Salinity is one of the extrinsic factors that directly affect fish body metabolism and the osmoregulation processes. The body weight of freshwater fishes decreases when exposed to higher salinity due to their hypertonic body fluids being lower compared to the external medium, hence, energy utilization, feed consumption, growth, and survival are significantly affected (Tort & Teles 2011). Therefore, the present study aims to evaluate the effect of dietary benfotiamine on the growth and survival of African catfish juveniles. Also, since salinity is a key abiotic factor limiting freshwater species, it is critical to investigate their responses to abrupt shift to hypersalinity to determine whether dietary benfotiamine could enhance *C. gariepinus* resistance.

Material and Method

Experimental animals. The study was carried out at the laboratory of the National Institute of Molecular Biology and Biotechnology (NIMBB), University of the Philippines Visayas, Miagao, Iloilo, Philippines. Approximately 450 healthy catfish fry were procured from Zarraga, Iloilo, Philippines. Upon arrival, the experimental fish was disinfected and acclimatized to laboratory conditions, with a water temperature of 27^{o}C and 0 ppt water salinity for 7 days. Samples were distributed to 9 experimental tanks (50 fry tank^{-1}) containing 40 L of filtered freshwater with constant aeration. The test animals were fed with the control diet prior to the feeding trial examination.

Experimental feed preparation. Three diets were formulated. The first diet contained 5% carbohydrate (control variable). The second diet contained 20% carbohydrate, whereas the third diet contained 20% carbohydrate supplemented with 0.02% benfotiamine. The amounts of corn starch, soybean oil, vitamin mix and benfotiamine in the experimental diets were adjusted by replacing an equal amount of carboxymethylcellulose (CMC). The composition of diets and proximate analysis are presented in Table 1.

Table 1

Composition of experimental diets fed to *Clarias gariepinus*

	Feeds (% composition)		
Feed ingredients	*Control*	*HC*	*HCB*
Fish meal	48.59	48.59	48.59
Soybean	14.69	13.69	13.69
Rice bran	10.37	10.37	10.37
Cornstarch	15.00	20.00	20.00
Soybean oil	3.00	2.00	2.00
Vit mix	3.50	2.00	2.00
CMC	4.85	3.35	3.33
Benfotiamine	0.00	0.00	0.02
Total	100.00	100.00	100.00
Proximate analysis	*Control*	*HC*	*HCB*
Moisture	10.08	13.73	11.99
Crude protein	44.08	44.60	42.84
Crude lipid	3.89	3.29	3.97
Crude ash	11.26	9.80	10.08
Crude fiber	2.94	2.36	1.88
Energy (kcal kg^{-1})	322.33	312.89	324.05

Note: HC - 20% carbohydrate; HCB - 20% carbohydrate with benfotiamine; CMC - carboxymethylcellulose.

Attractability assessment. The attractability test was conducted by using a rectangular glass tank (Nudalo et al 2020) shown in Figure 1. Three rectangular glass tanks were constructed, 90x30x30 cm in length, width and height, and consisted of three major chambers. The first chamber was used for acclimatization, the second chamber served as their entrance, and the third chamber was divided into three sub-chambers for the three test diets. The three sub-chambers were provided with an opening to allow the fish to access the feeds.

Approximately 30 individuals of catfish fry were fasted for 12 h in a separate container. 10 fry were randomly selected from each dietary treatment and were placed in the acclimatization chamber for 1 h. The chamber was separated from the next chamber by a liftable shutter. 2 g of each of the three experimental diets were placed in each feeding chamber (A, B, C) for 10 min, after which the shutter was lifted for the fry to access the diets. Attractability of the diet was calculated as the proportion of fish at a given diet at 1 min, 5 min, and 10 min following shutter lifting.

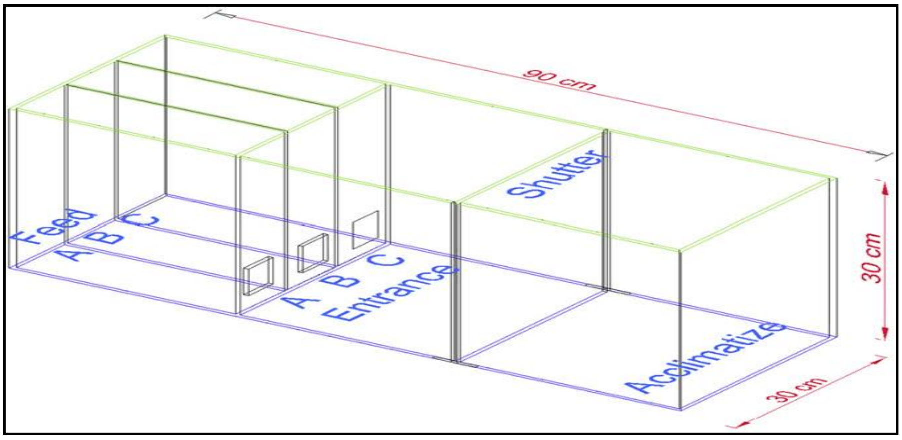

Figure 1. Rectangular glass tank used for attractability test (Suresh et al 2011).

Feeding trial. 450 healthy catfish fry with an initial average body weight of 0.081 g were randomly distributed in the experimental tanks containing 40 L of water, with salinity of 0 ppt, set in a closed recirculating system. The recirculating system was composed of a water pump that delivers water right into the elevated physical filter container with sand and gravel. Water from the physical filter flowed by gravity through the biological filter in the next level. This container consisted of disinfected dead oyster shells. From the biological filter, water was supplied again to the experimental tanks at a constant flow rate of 13.3 L h^{-1}.

The three dietary treatments were fed to three replicate groups of catfish for 60 days. The experimental fish were fed at the rate of 30% body weight initially for the first 15 days and the rate was adjusted to 20, 15, 10% in 30, 45, and 60 days, respectively. Feeding was performed thrice daily (08:00, 12:00, and 16:00). Prior to feeding, accumulated uneaten feed and organic waste were siphoned to maintain water quality, followed by replenishing the water. About 30% of the total water volume in the closed recirculating system was changed every week. Subsequently, net shelters and the dried leaves of tropical almond (*Terminalia catappa*) were placed in every container to lessen aggression among large-size catfish, while enhancing water condition. Formulated diets used were crushed into ~0.25 mm particles, adjusted into slightly bigger sizes after 30 days.

Growth parameters. Sampling was conducted every 15 days by bulk weighing of the fish in each replicate. For the growth measurement of the experimental samples, growth indicators such as weight gain (WG), specific growth rate (SGR), feed intake (FI), survival rate (SR), and feed conversion ratio (FCR) were determined. The formulas are presented below.

Weight gain, WG (g) = Final average body weight (ABW) – Initial ABW

Specific growth rate (SGR) = 100*[Ln (Final ABW g) – Ln (Initial ABW g)]/ Number of days

Feed intake (FI) g = Sum of daily feed weight

Feed conversion ratio (FCR) = Total feed intake / Weight gain

Survival rate (%) = 100*(Total no. of live catfish)/ Total no. of catfish stocked

Lethal concentration (LC50). The LC50 (median lethal concentration) for salinity was determined in an experimental set up. Groups of *C. gariepinus* fry, 10 fish per container, were exposed to 0, 4, 8, 12, and 16 ppt salinity for 96 hours. At 24, 48, 72 and 96 h, dead fish were counted in the different salinities along with the control group. The data were assessed according to Behrens-Karber’s method using the following formula (Klassen 1991):

96 h LC50 (ppt) = LC100 $\sum A \text{x} B/N$

Where: LC50 and LC100 indicate the lethal doses for 50% and 100% of the tested fish; *A* represents the differences between two consecutive doses; *B* is the arithmetic mean of the mortality caused by two consecutive doses; and *N* is the number of tested fish in each group.

Abrupt shift to higher salinity. At the end of the 60-day feeding trial, a total of 10 representative samples in each replicate were randomly and abruptly subjected to higher salinity 10 ppt ($^{o}/_{oo}$) for 96 h.

To assess survival, the experimental samples were defined as dead when no spontaneous movement and lack of response to prodding with a glass rod was observed. The number of dead samples were monitored every 12 h until the termination of the challenge test. The percentage of mortality was determined by using the following formula:

Mortality rate (%) = 100*(Dead fry count / Initial fry count)

Statistical analysis. Data on growth parameters such as final average body weight (FABW), weight gain (WG), specific growth rate (SGR), feed intake (FI), survival rate (SR), and feed conversion ratio (FCR) were analyzed using Statistical Package for the Social Sciences (SPSS) software, Version 20. Each parameter was tested for normality of distribution and homogeneity of variances before proceeding to one-way ANOVA with Duncan's Multiple Range Test (DMRT) as the post-hoc test. Parameters in different experimental groups were compared ($p<0.05$) and data is presented as mean ± standard error of the mean (SEM).

Results

Attractability assessment. The attractability of the experimental diets to *C. gariepinus* fry is presented in Table 2. Initially, no significant differences among the test diets were observed (i.e., at 1 min). However, after 5 min, the HC diet exhibited the lowest percentage of attractability, while no significant differences were observed between the control and HCB diets. The HCB diet resulted in significantly the highest attractability among other diets at 10 min.

Table 2

Percentage of *Clarias gariepinus* attracted to the test diets

Test diet	*1 min*	*5 min*	*10 min*
Control	$0.16±0.33^{a}$	$0.16±0.03^{a}$	$0.20±0.00^{b}$
HC	$0.13±0.33^{a}$	$0.10±0.00^{b}$	$0.13±0.03^{c}$
HCB	$0.16±0.33^{a}$	$0.20±0.00^{a}$	$0.30±0.00^{a}$

Note: HC - 20% carbohydrate; HCB - 20% carbohydrate with benfotiamine; values are mean ± SE for three replicates; mean values in the same row with different superscripts are statistically different ($p<0.05$).

Growth and feed efficiency. After 60 days of feeding, the HCB diet resulted in significantly higher values than the C and HC diets in terms of FABW, WG, FI, and SGR (Table 3). Catfish juveniles fed with HCB gained the lowest FCR, of 0.71, followed by the control (1) and HC (1.32). The HCB diet resulted in significantly higher SR, followed by the control and HC.

Table 3

Growth parameters, feed efficiency, and survival rate of the *C. gariepinus* juvenile fed with experimental diets within a 60-day feeding trial

Diets	*IABW (g)*	*FABW (g)*	*WG (g)*	*SGR (% bw day^{-1})*	*FI (g)*	*FCR*	*SR (%)*
C	0.081	$0.76±0.02^{b}$	$29.90±0.99^{b}$	$4.55±0.13^{b}$	$30.00±1.73^{b}$	$1.01±0.08^{b}$	$89.33±1.76^{a}$
HC	0.081	$0.61±0.03^{c}$	$21.64±1.53^{c}$	$3.57±0.19^{c}$	$28.50±2.59^{b}$	$1.32±0.09^{a}$	$83.33±1.76^{b}$
HCB	0.081	$1.44±0.02^{a}$	$60.80±1.54^{a}$	$9.06±0.10^{a}$	$43.50±2.29^{a}$	$0.71±0.03^{c}$	$90.00±1.15^{a}$

Note: C - control; HC - 20% carbohydrate; HCB - 20% carbohydrate with benfotiamine; IABW - initial average body weight; FABW - final average body weight; WG - weight gain; SGR - specific growth rate; FI - feed intake; FCR - feed conversion ratio; SR - survival rate; values are mean ± SE for three replicates; mean values in the same row with different superscripts are statistically different ($p<0.05$).

Determination of median lethal concentration (LC50) for abrupt salinity exposure. Prior to the challenge of abrupt shift to higher salinity, 96 h LC50 was determined experimentally and was calculated to be at 10 ppt. This salinity level was then used for the experiment on abrupt change to higher salinity challenge test.

Table 4

Mortalities and 96h LC50 of salinity in the African catfish (*Clarias gariepinus*) fry according to Behrens-Karber's method (Klassen 1991)

Salinity (ppt)	*No of exposed fish*	*No of dead fish*				*Overall*	*A*	*B*	*AxB*
		D1	*D2*	*D3*	*D4*				
0	10	0	0	0	0	0	0	0	0
4	10	0	0	0	0	0	4	0	0
8	10	0	0	0	0	0	4	0	0
12	10	4	2	10	10	10	4	5	20
16	10	10	10	10	10	10	4	10	40
									Σ=60

Note: 96 h LC50 = 10.0 ppt; A - differences between the two consecutive doses; B - arithmetic mean of the mortality caused by two consecutive salinity levels.

Abrupt shift to higher salinity. Results on a 96 h abrupt salinity challenge test of the experimental fish are presented in Table 5 and Figure 2. No mortality was recorded at 12 h of exposure at 10 ppt. However, at 24 h, the HCB group significantly exhibited the lowest cumulative mortality rate of 56.66%, whereas 100% and 93.33% were recorded in HC and C groups, respectively ($p<0.05$). At 36 h, a cumulative mortality of 100% was recorded in HC group. The HCB group maintained a cumulative mortality of 56.66% from 36 h onwards, until termination.

Table 5

Mean cumulative mortality of *Clarias gariepinus* after 96 h abrupt shift to higher salinity (10 ppt)

Treatments	*Survival of fry (%)*			
	12 h	*24 h*	*36 h*	*48 h*
Control	00.00±0.00	93.33±3.33[a]	100±0.00[a]	100±0.00[a]
HC	00.00±0.00	100±0.00[a]	100±0.00[a]	100±0.00[a]
HCB	00.00±0.00	56.66±6.66[b]	56.66±6.66[b]	56.66±6.66[b]

Note: HC - 20% carbohydrate; HCB - 20% carbohydrate with benfotiamine; values are mean ± SE for three replicates; mean values in the same row with different superscripts are statistically different ($p<0.05$).

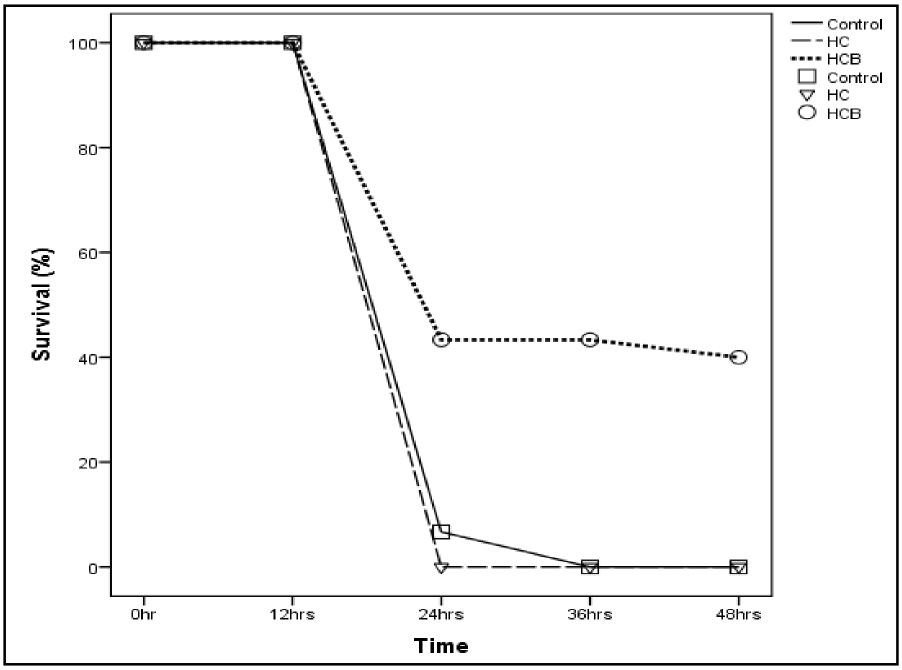

Figure 2. Cumulative mortality of *Clarias gariepinus* fry subjected to abrupt shift to higher salinity (0 ppt to 10 ppt) for 96 h.

Discussion. In formulating an experimental diet, attractability and palatability are the primary aspects that need assessment to determine whether the test samples are effectively able to accept it. Better response to the diets greatly affects consumption as well as digestibility leading the fish to improve in FI, FCR, and growth (Carr 1988; Bowker 2013). Fish are able to detect and recognize substances. Hence, regardless of what sensory organs are involved in feeding, attractiveness is responsible for the fish selecting their food preferences (Lokkeborg et al 2014; Morais 2016; Olsen & Lundh 2016). Dworjanyn et al (2007) noted that the most inexpensive method in achieving optimum growth, survival and gonadal index is to increase the feed attractability and palatability. Palatable and attractable diets lead to minimizing waste from uneaten feed, reducing feed cost, and alleviating water quality from rapid deterioration, thus resulting in successful fish farming. In the present study, the benfotiamine-supplemented high carbohydrate diet showed more attractability compared to the C and HC diets for *C. gariepinus* fry. According to previous studies, thiamine or vitamin B1 consists of a pyrimidine ring and thiazole ring associated with a methylene bridge in their structure, during reaction between sugar contraction and proteins (known as Maillard reaction). The thiazole ring generates aromas and meaty flavors when it begins to breakdown (Buttery et al 1984; Sasanam et al 2022), making the diet more attractable to *C. gariepinus.*

The formulated benfotiamine-supplemented high carbohydrate diet demonstrated a significant improvement in terms of growth, feed utilization, and survival of African catfish juveniles relative to the high carbohydrate-only diet and to the control diet. This observation is in agreement with those for the blunt snout bream, which elicited best growth performance when fed with HCB (1.425 mg kg^{-1}) (Xu et al 2017). Moreover, the same study inferred an enhanced higher value in terms of whole body lipid, tissue glycogen, and plasma insulin levels contributing to the beneficial effects to fish health conditions, thus attaining higher survival. Nudalo et al (2020) reported that *P. monodon* exhibited significantly higher growth performance following feeding with HCB than those fed with C and HC. In addition, at the termination of their feeding trial, shrimps were challenged with ammonia sublethal toxicity. As a result, the HCB diet significantly enhanced the shrimps' resistance against ammonia toxicity. In the present study, *C. gariepinus* juveniles fed with HCB diet exhibited significantly higher values of FABW, SGR, FI, and WG than those fed the C and HC. Among the three diets, the HCB group exhibited significantly the lowest FCR value, demonstrating that benfotiamine could enhance feed efficiency, i.e. efficient conversion of food nutrients into energy and/or intermediary substrates for growth.

One of the unique features of benfotiamine is the present of the open thiazole ring, which makes the pro-vitamin B1 highly lipid-soluble and enables it to enter directly through the cell membrane resulting in higher bio-absorption and bio-availability as compared to thiamine (vitamin B1). Its active form is thiamine pyrophosphate (TPP), which is responsible for providing pentose sugar for nucleotide synthesis and also it is vital for the redox homeostasis (Catacutan & De la Cruz 1989). In the present study, *C. gariepinus* fed with HCB diet exhibited significantly the highest SR among the experimental diets.

As for the HC group, values for experimental *C. gariepinus* fry were all significantly lower than those in the C and HCB groups. This might be due to the observed low FI, which subsequently resulted in growth retardation. According to previous studies, an intake of HC diet alone might cause persistent hyperglycemia, which is considered to induce physiological stress in response to metabolic activities, therefore significantly affecting the growth performance of blunt snout bream (Xu et al 2017), similar to the observation in the present study. In addition, the liver of *M. amblycephala* showed an induced inflammation associated with low antioxidant ability caused by high dietary carbohydrate intake (Xu et al 2017).

Thiamine deficiency can cause an induced congestion of the skin and fins, atrophy of intestine and hepatopancreas, decrease the activities of the digestive system leading to poor growth and survival of juvenile Jian carp (*Cyprinus carpio* var. *Jian*) (Huang et al 2011). The same researchers reported that intestinal digestive enzymes and increase of feed efficiency of juvenile Jian carp (*C. carpio* var. *Jian*) were enhanced after optimal supplementation of benfotiamine. In mammals, inhibition of inflammation caused by hyperglycemia-induced metabolic damages were reduced by using the benfotiamine supplementation (Hammes et al 2003; Serhiyenko et al 2019).

The increase in the level of salinity results from the increased evaporation rates, sea-level rise limiting the freshwater boundaries, among other things. Salinity level variations and corresponding environmental changes define the oxidative stress physiology in aquatic aerobic animals. Oxidative stress is generated when imbalance in the production and neutralization of active oxygen species is moved in favor of the former (Loro et al 2012). The active oxidants or free radicals such as superoxide radicals, hydroxy radicals and H_2O_2 derived from oxygen are collectively called reactive oxygen species (ROS). Salinity and some biotic factors that exist in the habitat of the organism affect its growth and development, as well as oxidative stress (Kim et al 2001; Boeuf & Payan 2001). As to the possible modulating effects of benfotiamine on ROS, Goncalves et al (2019) showed that the antioxidant effect of dietary benfotiamine in tissues resulted in increased metabolic activity during exercise. In addition, these researchers observed that benfotiamine reduced lipid peroxidation and protein damage in muscles, and it also increased and preserved antioxidants. Benfotiamine prevents lipopolysaccharide-induced generation of ROS and macrophage death in vitro (Yadav et al 2010). The stated role of benfotiamine in several studies offers an explanation of the mechanism by which a decreased rate of mortality was observed in the present study after the abrupt exposure to hypersalinity.

Conclusions. In conclusion, a benfotiamine-supplemented high carbohydrate diet (HCB) significantly improved diet attractability, growth performance, feed utilization, and survival. In addition, the HCB diet improved the resistance of the *C. gariepinus* to abrupt shifts to higher salinity.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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Authors:
Rey Lindayao Obeda, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao 5023 Iloilo, Philippines, e-mail: rlobeda@up.edu.ph
Barry Leonard Tumbokon, UPV National Institute of Molecular Biology and Biotechnology, University of the Philippines Visayas, Miagao 5023 Iloilo, Philippines, e-mail: aerumser@yahoo.com
Augusto Serrano Jr., Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao 5023 Iloilo, Philippines, e-mail: serrano.gus@gmail.com

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